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Impact of microwave pre-treatment on the batch anaerobic digestion of two-phase olive mill solid residue: a kinetic approach

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SUMMARY: The effect of a microwave (MW) pre-treatment on two-phase olive mill solid residue (OMSR) or alperujo with a view to enhancing its anaerobic digestibility was studied. The MW pre-treatment was carried out at a power of 800 W and at a targeted temperature of 50 °C using different heating rates and holding times. The following specific energies were applied: 4377 kJ·kg TS^{-1} (MW1), 4830 kJ·kg TS^{-1} (MW2), 7170 kJ·kg TS^{-1} (MW3) and 7660 kJ·kg TS^{-1} (MW4). The maximum methane yield, 395±1 mL CH_4·g VS_{added}^{-1}, was obtained for MW4. The effect of the pre-treatment on the kinetics of the process was also studied. The methane production curves generated during the batch tests showed a first exponential stage and a second sigmoidal stage for all the cases studied. In the first stage, the kinetic constant for the pre-treatment MW1 was 54.8% higher than that obtained for untreated OMSR.

KEYWORDS: *Alperujo; Anaerobic digestion; Batch; Kinetics; Microwave*

RESUMEN: ***Impacto del pretratamiento con microondas sobre la digestión anaerobia en régimen discontinuo de residuos sólidos de almazaras de dos fases: un enfoque cinético.*** El efecto del pretratamiento con microondas (MW) sobre el residuo semisólido procedente de la elaboración del aceite de oliva por el sistema de dos fases o alperujo fue estudiado con el objeto de aumentar su digestibilidad anaerobia. El pretratamiento fue llevado a cabo a una potencia de 800W y a una temperatura de 50 °C empleándose distintas velocidades de calentamiento así como diferentes tiempos de espera para obtener dichas condiciones. Las siguientes energías específicas fueron aplicadas: 4377 kJ·kg TS^{-1} (MW1), 4830 kJ·kg TS^{-1} (MW2), 7170 kJ·kg TS^{-1} (MW3) y 7660 kJ·kg TS^{-1} (MW4). El máximo rendimiento 395±1 mL CH_4·g $SV_{añadidos}^{-1}$ se obtuvo para MW4. El efecto del pretratamiento en la cinética del proceso también fue estudiado. Las curvas de producción de metano durante los ensayos mostraron una etapa exponencial y una sigmoidal en todos los casos. En la primera etapa, la constante cinética para MW1 fue 54.8% mayor que la obtenida para el alperujo sin pretratar.

PALABRAS CLAVE: *Alperujo; Cinética; Digestión anaerobia; Microondas; Régimen discontinuo*

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1. INTRODUCTION

Olive oil production is one of the most important agro-industrial activities in the Mediterranean area (García-Sánchez *et al.,* 2014). 90% of Spanish olive oil factories currently use the two-phase olive oil milling technology. This technology generates a complex aqueous solid residue from the primary centrifugation step, commonly called olive pomace, *alperujo*, olive mill solid residue (OMSR) or olive mill solid waste. 800 kg of OMSR are produced per tonne of olives milled (Rincón *et al.,* 2008a, 2008b, 2009). The average composition of the OMSR is: water (60–70%), lignin (13–15%), cellulose and hemicellulose (18–20%), olive oil retained in the pulp (2.5–3%), and mineral solids (2.5%) (Rincón *et al.,* 2013a). Therefore, OMSR has a high organic matter concentration leading to an elevated polluting load. The high level of pollution and large volumes of solid residues generated (more than 2 million tonnes per year in Spain) create serious environmental problems, especially when taking into account the 2,000 existing Spanish olive oil factories, most of which are located in the south of Spain (Borja *et al.,* 2006).

Anaerobic digestion (AD) of solid wastes is an efficient, attractive and well-established option for solid waste treatment because of its excellent waste stabilization and high energy recovery (Page *et al.,* 2014). The AD of particulate materials and complex compounds is carried out by anaerobic microorganisms (Rincón *et al.,* 2013b) and it occurs in four major steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is generally the rate-limiting step when bacteria release extracellular enzymes that break down and further solubilize organic particulate matter to be used as substrate in subsequent reactions (Jackowiak *et al.*, 2011). Therefore, to improve digestion efficiency, the most productive approach is to disrupt the chemical bonds in the material to be subjected to hydrolysis. In fact, the structure and composition of lignocellulosic compounds makes its AD especially difficult (Hendriks and Zeeman 2009). In addition, the presence of lignin in this structure acts as a physical barrier that induces a non-productive adsorption of the enzyme. Thus, with a view to disrupting the lignocellulose structure of the wastes in order to increase its anaerobic digestibility, pre-treatment would appear to be not only ideal but also necessary in some cases. The efficiency of a pre-treatment can be evaluated by the generated matter solubilization, the increase in anaerobic biodegradability and its cost.

Pre-treatment by irradiation with microwaves is one of the methods recently reported in the literature for improving the anaerobic biodegradability of different lignocellulosic, complex wastes and biomass (i.e. wheat straw, microalgae biomass, organic fraction of municipal solid waste, food industrial sewage sludge, etc.) (Beszédes *et al.,* 2011; Jackowiak *et al.,* 2011; Li *et al.,* 2012; Passos *et al.,* 2013; Sapci *et al.,* 2013; Shahriari *et al.,* 2012) as well as of primary and secondary waste activated sludges (WAS) (Eskicioglu *et al.,* 2007; Zheng *et al.,* 2009). Microwaves can improve the rupturing of the cell walls and complex compounds, thus enhancing anaerobic hydrolysis in two different ways (Solyom *et al.,* 2011). Firstly, the thermal effect corresponds to degradation caused by temperature increase. The internal heating and evaporation of the intracellular water causes an increase in internal pressure which in turn can cause the cell wall to rupture. Furthermore, in microwave (MW) pre-treatment energy is supplied by an electromagnetic field directly to the material and this leads to rapid heating throughout the material thickness with reduced thermal gradients. Volumetric heating can also reduce processing times and save energy. Secondly, the so-called 'a-thermal' effect must also be considered. This occurs when the alternating electric field of microwaves is capable of forcing the polarized side chains of the cell wall macromolecules to break their hydrogen bonds, and, thus, alter their structure (Eskicioglu *et al.,* 2007; Jackowiak *et al.,* 2011).

Therefore, MW pre-treatment is an alternative method for conventional heating and could potentially yield better results than classical thermal pre-treatment. However, some temperatures could also favor the generation of certain phenolic compounds and furan derivatives which are undesirable as they are inhibitors of anaerobic microorganisms (Taherzadeh and Karmini, 2008). To sum up, operating conditions must be determined for each specific waste so as to optimize microwave pre-treatments.

Based on the existing knowledge about pre-treatments, including the scarce information reported about the pre-treatment of OMSR, the aim of the present study was to evaluate the MW pre-treatment of *alperujo* using a power of 800 W and a targeted temperature of 50 °C on its anaerobic biodegradability through biochemical methane potential tests. The influence of the heating rate, ramp time, holding time at targeted temperature and, therefore, of the specific energy applied were also assessed. The effect of the above-mentioned MW pre-treatment conditions on the kinetic constants of the anaerobic process and ultimate methane yield were evaluated in BMP tests carried out at the mesophilic temperature (35 °C).

2. MATERIALS AND METHODS

OMSR was collected from a two-phase olive oil mill, located in the *Instituto de la Grasa* (*CSIC*), Seville, Spain. The OMSR was sieved through a 2 mm mesh to remove olive stone pieces before characterization and being put to use. The main characteristics of OMSR were: pH=4.9±0.2; total chemical

oxygen demand, CODt=331±1 g $O_2 \cdot kg^{-1}$; soluble chemical oxygen demand,CODs=143±3 g $O_2 \cdot kg^{-1}$; total alkalinity, TA=2.5±0.1 g $CaCO_3 \cdot kg^{-1}$; total solids, TS=265±3 $g \cdot kg^{-1}$; volatile solids, VS=228±2 $g \cdot kg^{-1}$; ammoniacal nitrogen, AN=0.3±0.0 g ammoniacal $N \cdot kg^{-1}$; hemicellulose=11.3±0.2%; cellulose=5.2±0.1%; lignin=19.7±0.4%; and fat=3.8±0.3%.

A laboratory Microwave Accelerated Reaction System "Mars 5" (CEM Corporation Matthews, North Carolina) was used to pre-treat the OMSR. Samples were heated to a temperature of 50 °C using a constant frequency of 2450 MHz and a power of 800 W. To achieve this temperature, ramp times of 2.5 and 10 min at heating rates of 5 and 20 $°C \cdot min^{-1}$, respectively, were used. Once the target temperature was achieved, it was kept for 1, 5 and 10 min in four different combinations, achieving applied specific energies or energy inputs of 4377, 4830, 7170 and 7660 kJ·kg TS^{-1}. Table 1 shows the different conditions studied: MW1, MW2, MW3 and MW4. To minimize losses, samples were cooled down to room temperature before opening.

The anaerobic experiments were run in 250 mL reactors. These reactors were continuously stirred at 500 rpm and maintained at 35±2 °C. An inoculum:substrate ratio of 2 was used (on a VS basis). A trace element solution was also added to each digester, a detailed description of this solution is given elsewhere (Rincón *et al.,* 2010). Two reactors with no added substrate were used as controls. Each experiment was carried out in duplicate.

Biogas was passed through a 3N NaOH solution to capture CO_2 on the assumption that the remaining gas was methane. The AD experiments were run until the last day production was lower than 2% of the accumulated methane produced.

The inoculum used in the BMP assays was obtained from an industrial anaerobic reactor (UASB) treating brewery wastewater at a mesophilic temperature. The characteristics of the anaerobic inoculum used were: pH: 7.5 and VS: 22.2 $g \cdot L^{-1}$.

Fat was analyzed by the official EEC method No 2568/91 (EEC Official Diary, L248/1 of 05.09.1991). Cellulose, lignin and hemicellulose were determined according to the van Soest method (Van Soest *et al*., 1991). Total chemical oxygen demand (CODt) was determined as described in Rincón *et al*., (2013a). CODs, AN, TS and VS were determined according to the Standard Methods (APHA, 1998). Specifically, CODs was determined by the method 5220D. TS and VS were determined by the methods 2540B and 2540E, respectively. A pH-meter model Crison 20 Basic was used to analyze pH. TA was determined by pH titration to 4.3.

3. RESULTS AND DISCUSSION

The degree of chemical oxygen demand solubilized was calculated by Eq. 1: where CODs is the soluble chemical oxygen demand measured after each pre-treatment condition tested and CODt, the total initial COD of the OMSR (Carrère *et al.*, 2009):

$$\text{COD solubilization (\%)} = (\text{CODs}/\text{CODt}) * 100 \quad \text{(Eq. 1)}$$

COD solubilization values of between 41.1% and 43.1% were achieved after the different MW pre-treatments tested. Table 2 shows the characteristics of two-phase OMSR after the different MW pre-treatments in terms of VS, CODt, CODs and COD solubilization.

MW1 achieved the highest value, i.e. 43.1%. This value was slightly higher than that obtained for thermal pre-treated OMSR at 180 °C for 180 min (42%) (Rincón *et al*., 2013a). In this way, and in order to assess the 'a-thermal' MW effects for enhancing the anaerobic digestibility of WAS, a pre-treatment range of 50–96 °C, both using MW and conventional heating (CH) was carried out and reported in the literature. In the mentioned case, WAS samples resulted in similar particulate COD and biopolymer (protein and polysaccharide) solubilization and there was no discernable MW 'a-thermal' effect on the COD solubilization of WAS (Eskicioglu *et al.,* 2007).

In the present study, and within the narrow specific energy range applied (4337–7660 kJ·kg TS^{-1}) and low targeted temperature used (50 °C), there

Table 1. Operational conditions used in MW pre-treatment of OMSR: MW1, MW2, MW3 and MW4

Parameter (unit)	MW1	MW2	MW3	MW4
Power (W)	800	800	800	800
Targeted temperature (°C)	50	50	50	50
Ramp time (min)	10	2.5	10	2.5
Heating rate ($°C \cdot min^{-1}$)	5	20	5	20
Holding time at targeted temperature (min)	1	1	5	10
Applied energy per g of OMSR (kJ·g $OMSR^{-1}$)	1.16	1.28	1.9	2.03
Applied energy per g of TS (kJ·kg TS^{-1})	4377	4830	7170	7660

Table 2. Characteristics of the untreated OMSR and microwave pre-treated OMSR after the different pre-treatments used: MW1, MW2, MW3 and MW4

Parameter (unit)	Untreated OMSR*	MW1	MW2	MW3	MW4
CODs ($g \cdot kg^{-1}$)	143	142.65	138.14	136.05	137.7
Solubilization (%)		43.1	41.7	41.1	41.6
Volatile solids ($g\ VS \cdot kg^{-1}$)	228.00	227.89	233.03	223.94	221.02
Methane yield ($mL\ CH_4 \cdot g\ VS_{added}^{-1}$)	366±4	303±9	337±7	368±16	395±1

*CODt of the untreated OMSR: 331.1 $g \cdot kg^{-1}$. OMSR: olive mill solid residue. MW1 (specific energy: 4377 $kJ \cdot kg\ TS^{-1}$), MW2 (specific energy: 4830 $kJ \cdot kg\ TS^{-1}$), MW3 (specific energy: 7170 $kJ \cdot kg\ TS^{-1}$), and MW4 (specific energy: 7660 $kJ \cdot kg\ TS^{-1}$).

was no significant influence of this parameter on COD solubilization. Passos *et al.,* (2013) found that the main parameter influencing biomass solubilization was the application of high specific energies in the range of 21800–65400 $kJ \cdot kg\ TS^{-1}$ for MW pre-treatment of the microalgae biomass. MW irradiation of the primary sludge was found to increase the ratio of CODs/CODt from 2.5% to 6–7% for sludge with 4% TS concentration when the pre-treatment temperature increased from 35 °C to 90 °C (Zheng *et al.,* 2009). In the MW pre-treatment of food industrial waste, solubility increased from 9.7% to more than 40% when the MW intensity rose from 0.5 to 5 $W \cdot g\ BOD_5^{-1}$ (Beszédes *et al.,* 2011). In the same way, an increase in the CODs/CODt ratio from 9.4% to 13.8% in the MW pre-treatment of switchgrass when the temperature increased from 90 °C to 180 °C in order to enhance its anaerobic digestibility has recently been reported (Jackowiak *et al.,* 2011). However, these temperatures were much higher than the 50 °C used in the present study.

As can be seen in Table 2, the methane yields obtained after 20 days of digestion were 303±9, 337±7, 368±16, and 395±1 mL $CH_4 \cdot g\ VS_{added}^{-1}$ for pre-treated OMSR at MW1, MW2, MW3 and MW4 conditions, respectively, and 366±4 mL $CH_4 \cdot g\ VS_{added}^{-1}$ for untreated OMSR. Figure 1 shows the variation in the specific methane yield (B), against time for the tests carried out with untreated OMSR and MW pre-treated OMSR for the different specific energies used (MW1, MW2, MW3 and MW4).

Only MW4 led to a slight increase of 8% in the methane yield with respect to the value obtained for the untreated substrate. In addition, an increase was observed in the methane yield to increased applied specific energy, and the maximum value (395±1 mL $CH_4/g\ VS_{added}^{-1}$) was obtained for the maximum specific energy tested (MW4).

The highest methane yield obtained in the present study at the MW4 condition was of the same order of magnitude as that obtained in BMP tests of thermal pre-treated OMSR at 120 °C for 180 minutes (392±14 mL $CH_4 \cdot g\ VS_{added}^{-1}$) and 4% higher than that reached for thermal pre-treated OMSR at 180 °C for 180 minutes (380±1 mL $CH_4 \cdot g\ VS_{added}^{-1}$) (Rincón *et al.,* 2013a).

Some studies reported in the literature show an increase in the methane yield of MW pre-treated wastes as compared with the untreated ones. For instance, pre-treated MW WAS at a targeted temperature of 175 °C produced 31±6% more biogas than the untreated WAS by the 18th day of the BMP test (Toreci *et al.,* 2011). In this light, Eskicioglu *et al.* (2007) also reported a 16±4% increase in biogas production compared to a control for this same substrate after a MW pre-treatment at a temperature of 96 °C. BMP tests of the sludge from sequencing batch reactors subjected to MW pre-treatment at 85 °C showed a 16% increase in methane production as compared to untreated sludge. For other complex substrates, such as industrial food waste, specific biogas increased from 220 to 600 $mL \cdot g^{-1}$ when the energy applied in the MW pre-treatment increased from 0.5 to 5 $W \cdot g\ BOD_5^{-1}$ (Beszédes *et al.,* 2011). In BMP tests of microalgae biomass grown in wastewater, the final biogas yield increased from 12% to 78% when the substrate was pre-treated with MW at specific energy at an interval of 21,800–65,400 $kJ \cdot kg\ TS^{-1}$, higher values than those used in the present study (Passos *et al.,* 2013). In the same way, mesophilic batch AD experiments of the organic fraction of municipal solid waste pre-treated with MW at high temperatures showed a 4–7% improvement in biogas production over the untreated organic fraction of municipal solid waste (control) when the temperature increased from 115 °C to 145 °C. However, when this substrate was pre-treated at 175 °C, biogas production decreased due to the formation of refractory compounds inhibiting the digestion (Shahriari *et al.,* 2012).

Similarly to the present study, other researchers found no significant differences in methane yields between untreated and MW pre-treated samples. For example, the MW pre-treatment of switch grass at temperatures in the range of 90 °C to 180 °C induced no change in the ultimate volume of methane in BMP assays carried out for 42 days but had a positive effect on the reaction kinetics because the time required to reach 80% of ultimate methane volume was reduced by 4.5 days at 150 °C using the MW pre-treatment (Jackowiack *et al.,* 2011). In the same way, the low temperature (50–65 °C)

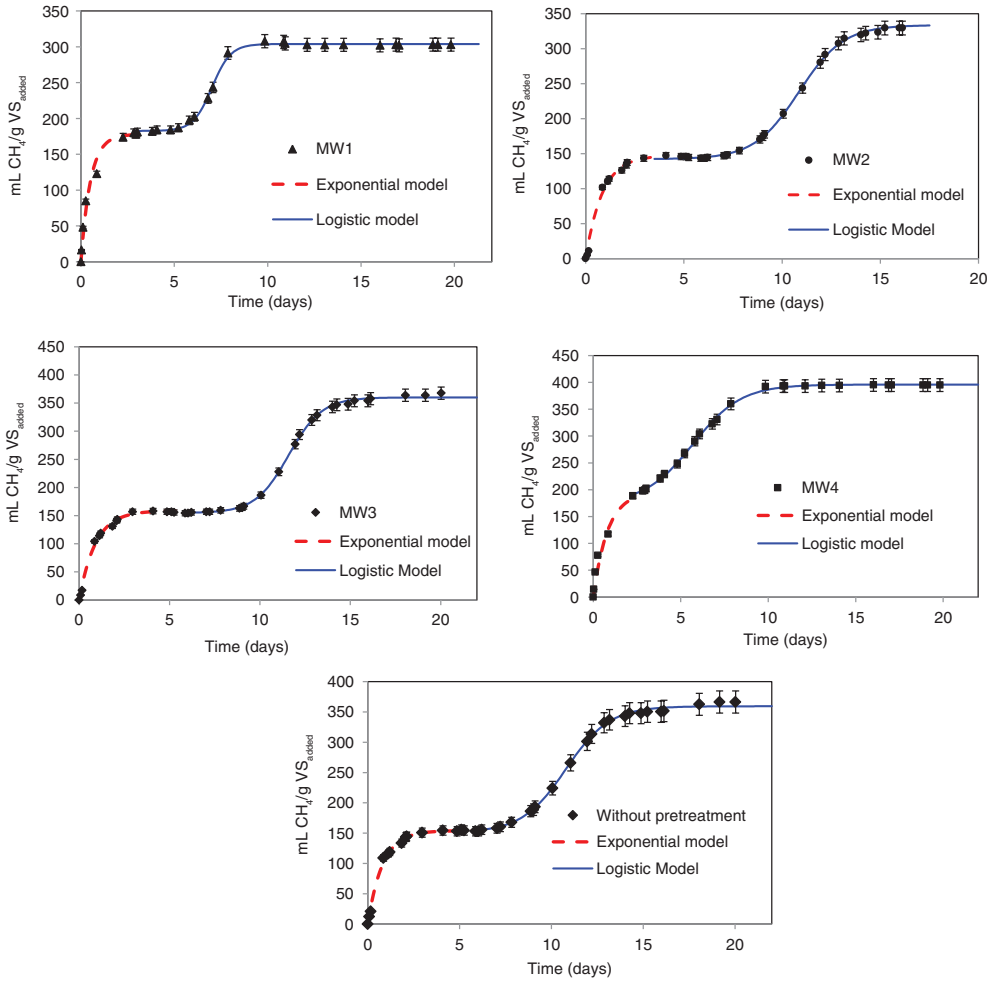

FIGURE 1. Cumulative methane yield, expressed as mL CH_4·g VS_{added}^{-1}, obtained during the BMP tests carried out with untreated OMSR and microwave (MW) pre-treated OMSR at the following operating conditions: MW1 (specific energy: 4377 kJ·kg TS^{-1}), MW2 (specific energy: 4830 kJ·kg TS^{-1}), MW3 (specific energy: 7170 kJ·kg TS^{-1}), and MW4 (specific energy: 7660 kJ·kg TS^{-1}).

MW pre-treatment of primary sludge (with 4% solids concentration) presented no obvious impact on sludge solubilization and ultimate biogas production in BMP tests of this pre-treated substrate carried out for 50 days (Zheng *et al.,* 2009). These temperatures were very similar to those assayed in the present study (50 °C). Finally, BMP assays of grass (Pennisetum hybrid) previously exposed to MW at 260 °C showed that the maximum production rate and total methane produced decreased by 18% and 12%, respectively, with respect to the values found for the untreated substrate (Li *et al.,* 2012).

The variation in the methane production with digestion time for untreated OMSR showed two different stages: a first stage of growth followed by a lag period, and a second stage in which the methane production rate increased gradually until the 20th day.

Similar behavior was observed for all the pre-treatments tested: MW1, MW2, MW3 and MW4. Two different models were used to explain this behavior:

A first-order exponential model for the first stage (Li *et al.,* 2012) given by Eq. 2:

$$B_1 = B_{max} \cdot [1 - \exp(-k \cdot t)] \qquad \text{(Eq. 2)}$$

where: B_1 (mL CH_4·g VS_{added}^{-1}) is the cumulative specific methane production, B_{max} (mL CH_4·g VS_{added}^{-1}) is the ultimate methane production, k is the specific rate constant or apparent kinetic constant ($days^{-1}$) and t (days) is the time.

And a sigmoidal model with its three characteristic phases, i.e. lag, exponential increase and final stabilization step was used for the second stage, i.e. between the 5th and 8th days and the last day of the operating period, i.e. the 20th day (Donoso-Bravo *et al.,* 2010; Li *et al.,* 2012):

$$B_2 = B_0 + P/[1 + \exp(-4 \cdot R_m \cdot (t - \lambda)/(P + 2))] \qquad \text{(Eq. 3)}$$

where: B_2 is the cumulative methane production during the second stage (mL CH_4·g VS_{added}^{-1}), B_0 is the cumulative methane production at the start-up of the second stage (mL CH_4·g VS_{added}^{-1}) and should approximately coincide with the value of B_{max} obtained at the end of the first stage, P is the maximum methane production obtained in the second stage (mL CH_4·g VS_{added}^{-1}), R_m is the maximum methane production rate (mL CH_4·g VS^{-1}·d^{-1}) and λ is the lag time (days).

Batch AD experiments of energy crops (i.e. maize, oat, ryegrass and wheat) at mesophilic temperature indicated that the rate of the conversion of these substrates into methane may first reach their maximum rate after several days of digestion and show that the cumulative methane production pattern from these crops followed a sigmoidal, rather than an exponential curve shape (Nielsen and Feilberg, 2012).

k and B_{max} for this first stage of methane production were calculated by the adjustment by non-linear regression of the pairs of experimental data B_1, t (Sigmaplot 11.0 software). The goodness of the fit of the experimental data to the model proposed for this first exponential stage is confirmed by the high values of the R^2 and the low values of the standard error of estimate (S.E.E.).

As can be seen in Table 3, the values of k obtained ranged between from 1.11±0.07 (MW3) to 1.95±0.16 $days^{-1}$ (MW1). k was higher for the MW1 pre-treatment at 4377 kJ·kg TS^{-1} of specific energy (k=1.95 d^{-1}) than for the other specific energies studied.

For pre-treatments MW2 and MW3, and for the untreated OMSR, k values were found almost in the same range, between 1.11 and 1.26 d^{-1}. Therefore, the kinetic constant for the MW1 pre-treatment was 64% and 75% higher than that obtained for the pre-treated OMSR at MW2 and MW3 conditions respectively, and 55% higher than for untreated OMSR. The highest value of k (1.95 $days^{-1}$) achieved for the MW1 pre-treated OMSR might be associated with its higher solubilization value (43.1%) achieved after pre-treatment, as compared to the other pre-treatment conditions.

In addition, the values of the kinetic constants obtained in the present research work for the MW pre-treated OMSR were higher than those obtained in BMP tests of thermal pre-treated OMSW at 120 °C and 180 °C for 180 minutes (Rincón *et al.,* 2013a).

During the first stage, B_{max} for pre-treatment MW4 was higher (196 mL CH_4·g VS_{added}^{-1}) than those obtained for the other MW pre-treatments assayed, whose values ranged between 148 mL

TABLE 3. Kinetic parameters obtained from the exponential and logistic models in the BMP tests of untreated OMSR and microwave pre-treated OMSR under MW1, MW2, MW3 and MW4 conditions

Exponential adjust				
	B_{max}	K		
Sample	(mL CH_4·g VS_{added}^{-1})	($days^{-1}$)	R^2	**S.E.E.**
Untreated OMSR	154±2	1.26±0.07	0.99	3.82
MW1	178±5	1.95±0.16	0.98	10.36
MW2	148±4	1.19±0.10	0.99	6.07
MW3	159±3	1.11±0.07	0.99	4.69
MW4	196±13	1.43±0.31	0.97	14.46

Logistic adjust						
	B_0	P	R_m	λ		
Sample	mL CH_4·g VS_{added}^{-1}	mL CH_4·g VS_{added}^{-1}	mL CH_4·g VS_{added}^{-1}·d^{-1}	**days**	R^2	**S.E.E.**
Untreated OMSW	151±2	208±2	44.0	10.8±0.0	0.99	3.5
MW1	183±1	121±2	61.0	7.0±0.0	0.99	2.95
MW2	142±1	192±2	42.7	10.8±0.0	0.99	2.42
MW3	155±1	205±2	56.2	11.6±0.0	0.99	3.04
MW4	174±5	222±5	40.5	5.7±0.1	0.99	2.76

B_{max} is the ultimate methane production, k is the specific rate constant or apparent kinetic constant, B_0 is the cumulative methane production at the start-up of the second stage, P is the maximum methane production obtained in the second stage, R_m is the maximum methane production rate and λ is the lag time. Parameters from the nonlinear regression fit: R^2: coefficient of determination; S.E.E.: standard error of estimate; OMSR: olive mill solid residue. MW1 (specific energy: 4377 kJ·kg TS^{-1}), MW2 (specific energy: 4830 kJ·kg TS^{-1}), MW3 (specific energy: 7170 kJ·kg TS^{-1}), and MW4 (specific energy: 7660 kJ·kg TS^{-1}).

CH_4·g VS_{added}^{-1} (MW2) and 178 mL CH_4·g VS_{added}^{-1} (MW1). These results might indicate a slight increase in easily biodegradable compounds after MW4 pre-treatment. However, a high percentage of complex substrates still exists, which diminished the degradation rate.

The logistic model has been previously used for estimating methane production in batch AD experiments of different substrates (Pommier *et al.,* 2007; Donoso-Bravo *et al.,* 2010; Li *et al.,* 2012; Nielsen and Fielberg, 2012). This model assumes the rate of methane production to be proportional to microbial activity (Altas, 2009).

For the logistic model, *P* obtained in the second stage had the highest value for pretreatment MW4 (222 mL CH_4·g VS_{added}^{-1}) followed by the untreated OMSR (208 mL CH_4·g VS_{added}^{-1}) and pretreatment MW3 (205 mL CH_4·g VS_{added}^{-1}) (Table 3). Moreover, when comparing the values of the R_m obtained in the logistic model, the optimum pre-treatment was MW1. Kinetics was the fastest for MW1 with 4377 kJ·kg TS^{-1} of specific energy and with 61.0 mL CH_4·g VS^{-1}·day^{-1} being produced, which is 39% higher than the value obtained for untreated OMSR and 43%, 9% and 51% higher than the values found at MW2, MW3 and MW4 conditions, respectively.

Li *et al.* (2012) also used a logistic function to assess the effect of a MW pre-treatment (at a maximum temperature of 260 °C and a power of 1,180 W) on grass (*Pennisetum hybrid*) in BMP tests. These authors found that the maximum methane production rate and total methane produced decreased by 18% and 12% respectively, compared with untreated raw material.

In the present study, the pre-treatment MW1 with 4377 kJ·kg TS^{-1} of specific energy most likely promotes the release of more easily biodegradable compounds, which allows an increase in the R_m and a decrease in the lag period.

The shortest lag phase (λ) was obtained for MW4, i.e. 5.7 days, while the longest lag phase was achieved for the untreated OMSR and MW3 pre-treated OMSR (i.e. 10.8 and 11.6 days, respectively). Long lag phases can lead to the generation of different inhibitor compounds that delay the start-up of the second phase in the methane production (Donoso-Bravo *et al.,* 2010). The maximum value of R_m achieved in the present work (61.0 mL CH_4·g VS^{-1}·day^{-1}) for the MW1 was somewhat lower that that obtained in BMP tests of OMSR previously treated thermally at 180 °C for 180 min (76.8 mL CH_4·g VS^{-1}·day^{-1}) (Rincón *et al.,* 2013a). However, the maximum value of R_m reached in the present study was much higher than that obtained in BMP tests of co-digestion mixtures of OMSR and *Dunaliella salina* with 75% OMSR-25% *D. salina* (48.1 mL CH_4·g VS^{-1}·day^{-1}) and 50% OMSR-50% *D. salina* (30.1 mL CH_4·g VS^{-1}·day^{-1}) (Fernández-Rodríguez *et al.,* 2014).

Taking into account that for pre-treated MW (MW1, MW2 and MW3) the methane yield values obtained were equal to or lower than the methane yield obtained for the untreated OMSR or control; there is no positive energy gain for these three cases. Therefore, for these cases the energy balance would clearly be negative and the MW pre-treatment would not be feasible from a practical point of view.

For the MW4 pre-treatment condition carried out with a specific energy of 7660 kJ·kg TS^{-1}, the methane yield (395 mL CH_4·g VS_{added}^{-1}) was 8% higher than that obtained for untreated OMSR (366 ml CH_4·g VS_{added}^{-1}). This assay corresponded to that performed to 800 W of power at a targeted temperature of 50 °C, using a heating rate of 20 °C·min^{-1} with a ramp time of 2.5 min and a holding time at the targeted temperature of 10 min. For these MW operating conditions the energy consumed or required for the MW pre-treatment was found to be 9.02 kJ·g VS^{-1}. The net energy resulting from the overproduction of methane was calculated by subtracting the methane volume of the untreated sample from that obtained from the MW pre-treated sample. In the present study, this was equal to + 1.04kJ·gVS^{-1}.

Therefore, a net balance between the consumed energy in the pre-treatment MW4, 9.02 kJ·g VS^{-1}, and the extra energy produced through BMP for the MW4, 1.04 kJ·g VS^{-1}, was found to be negative as the extra energy obtained through BMP was not enough to compensate the energy necessary for the pre-treatment.

Jackowiak *et al.* (2011) also reported a negative energy balance in batch AD experiments of wheat straw pre-treated with MW at much higher values than those used in the present study (maximum power of 1600 W and a targeted temperature of 260 °C). These same authors also reported that to obtain a positive energy balance, a MW device should consume less than 2.65 kJ·g VS^{-1}. In the same way, Houtmeyers *et al.* (2013) reported a negative energy balance during the semi-continuous AD of WAS previously subjected to a MW pre-treatment using a specific energy of 2100 kJ·kg $sludge^{-1}$, despite the fact that biogas production was increased by 27% as compared to untreated WAS.

4. CONCLUSION

Microwave pre-treatment of OMSR at specific energies applied in the range of 4377 (MW1)-7660 (MW4) kJ·kg TS^{-1} showed no significant influence on COD solubilization. MW4 led to an 8% increase in methane yield as compared to the untreated OMSR. Two stages, exponential and sigmoidal, were clearly differentiated in the BMP curves. The highest maximum methane production rate was found for the MW1. During the first stage, the kinetic constant for this pre-treatment was 55% higher than for untreated OMSR. During the second stage the maximum methane production rate (in the MW1) was 39% higher than that obtained for untreated OMSR.

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